

Tail-Docking Influences on Behavioral, Immunological, and Endocrine Responses in Dairy Heifers

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ABSTRACT

Behavioral and physiological changes were measured following tail-docking in primiparous heifers. One month before projected first parturition, 21 heifers were assigned to control (nondocked), docked, or docked with lidocaine groups. Heifers were banded to initiate tail-docking and the necrotic tail was removed after 144 h. Physiological, immunological, and behavioral measures were taken for 240 h following banding. Cortisol was not different for control and treated heifers. Haptoglobin increased for docked heifers by 168 h postbanding (24 h postdocking). α_1 -Acid glycoprotein decreased as haptoglobin increased, and α_1 -acid glycoprotein increased until 240 h postbanding. Tumor necrosis factor- α increased only with lidocaine and did not show an effect of docking by 240 h postbanding. Lymphocyte phenotyping demonstrated increased CD4⁺ and CD8⁺ peripheral blood mononuclear cells for docked plus lidocaine heifers and $\gamma\delta^+$ cells of those heifers tended to be reduced compared with docked heifers. Eating was the only maintenance behavior affected by banding in both docked groups (increased with banding and decreased with docking). The initial banding procedure did not alter heifer physiology and altered only eating behavior, but the cutting of the tail (docking) increased haptoglobin in response to the tissue damage and returned eating behavior to baseline. The use of lidocaine to anesthetize the tail before banding affected lymphocyte phenotypes and TNF- α (banding alone did not alter these parameters).

(**Key words:** tail-docking, behavior, physiology)

Abbreviation key: AGP = α_1 -acid glycoprotein, C = control, D = docked, DL = docked plus lidocaine, FITC

= fluorescein isothiocyanate, TNF- α = tumor necrosis factor- α .

INTRODUCTION

Tail-docking of dairy cattle is increasing in use by producers in the United States. Arguments supporting tail-docking include increased udder and milk hygiene, cleaner milking parlors and holding areas, and improved milker and handler comfort. The practice of tail-docking can also potentially be perceived as reducing the aesthetic value of cows and animal welfare. Besides the discomfort of the procedure, there is a possibility of chronic pain in the tail stump, altered ability of the cow to avoid flies during fly seasons, and reduced ability to use its tail for communication function (13) or self stimulation. Petrie et al. (20), found docking (application of a rubber ring) to be no more distressing, as indicated by plasma cortisol concentrations, than control handling with simulated docking in 3- to 4-mo old calves. However, Wilson (31) noted that tail movements were consistently greater 6 h after banding and that their tails had begun to swell by that time. By 24 and 48 h postbanding, tail movements in the banded animals were reduced compared with controls. On pasture, fly numbers increased on the rear legs of docked cows as did two fly avoidance behaviors specifically used on the rear legs, tail flicks and foot stamping (22). Docked cows on pasture also exhibited more fly avoidance behaviors than control cows (15). No research has been published to date on the effect of tail-docking on behavior or immune parameters in box stall, free-stall, or tie-stall housing typical in the United States. Our objectives in this study were to determine the effects of docking with and without lidocaine injections on behavior, endocrine, and immune measures after docking first calf heifers one month before calving while housed in box stalls.

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MATERIALS AND METHODS

Animal Care and Use

This study was reviewed and approved by the Purdue Animal Care and Use Committee. Animal housing and management was in accordance with "Guide for the Care and Use of Agricultural Animals used in Agricultural Research and Teaching" (1). Heifers were housed in iron box stalls (3.8×4.2 m) so that each heifer had access to another heifer through the bars of the pen. Each heifer had individual access to water and a complete balanced TMR. Heifers were placed in the stalls approximately 1 mo before expected calving. Heifers were acclimated to the new housing (for 1 wk) and to the chutes (once before banding) that would be used in the experiment. Several methods are used to dock tails of dairy cattle. When producers first dock their herds, many dock during the dry period (precalving for first calf heifers) to avoid affecting milk production. We chose the month before calving in first-calf heifers for that reason. Docking in young calves is usually done by banding alone; however, with older animals, a necrotic tail is also removed to keep the tail out of waste management systems. Because of these practices, we chose to use a procedure involving banding for approximately 1 wk followed by removal of the necrotic tail. Throughout this study we use the term banding for the first procedure and docking to refer to the removal of the necrotic tail.

Study Design and Sample Collection

Twenty-one primiparous Holstein heifers were blocked by expected calving date and randomly assigned to one of three treatments; control (**C**, non-banded and nondocked), docked (**D**, banded and docked), or docked with lidocaine (**DL**, banded with lidocaine and docked without lidocaine). On d 0, jugular blood was collected into heparinized tubes before treatment (0800 h). The heifers on the lidocaine treatment were given less than 5 ml of lidocaine (lidocaine hydrochloride injectable, 2%, Phoenix Pharmaceutical, Phoenix, AZ) subcutaneously, around the circumference of the tail with less than one ml at each site, just caudal to the selected banding site on the tail. Lidocaine administration and tetanus vaccination (Tetanus antitoxin, Fort Dodge Laboratories, Inc., Fort Dodge, IA) were performed under the direction of the herd veterinarian. A band castrator was used to apply a band on the tail level with the top of the udder and between two vertebrae. After obtaining a blood sample on d 6 (0800 h), tails were removed with clean shears at the banding site. Ten milliliters of jugular blood was collected at 1200, 1600, and 2000 h on d 0, at 0000, 0400, and 0800 h and

1600 h on d 1. On d 2, 3, and 4 samples were collected at 0800 and 1600 h. On d 6, 7, and 10 samples were collected at 0800 h only. An additional 20 ml of blood was collected on d 0, 1, 4, 7, and 10 at the 0800 h sampling time for leukocyte separation and analysis. All data are presented as hours postbanding.

Sample Analysis

All blood samples were refrigerated until spun at $700 \times g$ for 15 min. The plasma was removed and frozen (-70°C) for later analysis of acute phase proteins, cytokines, and cortisol. The buffy coat of each sample was used to separate peripheral blood mononuclear cells as previously described (5). Cells were suspended at 1×10^6 cells/ml in Rose Park Memorial Institute media 1640 (Sigma Chemical Co., St. Louis, MO). Aliquots (250 μl) were added to each of four tubes for phenotyping. The first tube was used as a control (cells only), the second tube for CD4 expression (cact138A, VMRD, Pullman, WA), the third tube for CD8 expression (cact80C, VMRD), and the fourth tube for TcR1 $\gamma\delta$ expression (86D, VMRD). Primary antibodies were mouse-anti-bovine and the secondary antibody was a fluorescein isothiocyanate (**FITC**) labeled rabbit anti-mouse IgG (Gibco, Grand Island, NY). The FITC fluorescence was used to measure lymphocyte markers by flow cytometry with a Coulter Elite flow cytometer (Hialeah, FL), using a 488-nm air-cooled argon laser for excitation and a 525-band pass for FITC labels.

αH_1 -Acid glycoprotein (**AGP**) was measured with radial immunodiffusion assay plates (Saikin Kagaku Institute Co., LTD, Sendai, Japan) and haptoglobin by ELISA (33). Cortisol was measured by a ^{125}I radial immunodiffusion assay (Coat-a-count, Diagnostic Products Corp., Los Angeles, CA). Tumor necrosis factor- α was measured with a biological assay using a WEHI 164 (American Type Culture Collection, Rockville, MD) cell line as previously described (14, 25).

Behavior Data Collection and Analysis

Heifer behavior was recorded by video camera (Panasonic AG-6740, Milpitas, CA) at a speed of one frame per 1.2 s (72-h mode) for 24 h pre- and postbanding and for 24 h pre- and postdocking (cutting the tail). Data were collected with a 5-min instantaneous scan sampling technique that had been validated with a continuous behavior sample. Behaviors included in the data were lying, standing, walking, drinking, eating, grooming, rubbing pen (cow to pen contact) including tail rubbing, cow-cow interactions, and head-toward-tail movement (Table 1).

Table 1. Description of maintenance behaviors and behaviors recorded to indicate discomfort following tail banding in dairy cows.

Activity	Description
Lying	Not supported by feet ¹
Standing	Supported by feet ¹
Walking	Movement of legs with a change in space
Drinking	Muzzle in water cup
Eating	Head in head-gate leading to feed bunk
Grooming	Licking a part of body, including self-grooming and allogrooming ²
Cow-cow interactions	Contactual behavior ²
Rub pen	Pressing some portion of the body against a blunt object and generating friction by moving one or the other while they are in sustained contact ²
Head-to-tail	Contact of the head (usually the tongue or nose) with the tail

¹From McGlone and Hellman (16).²Definition from Hurnik et al. (10).

Statistical Analysis

Physiological data were analyzed by the general linear model of SAS as a repeated measures design (3, 27). Means were separated using the protected least significant difference test when treatment, time, or treatment \times time effects were significant. Data are reported as least significant means. α_1 -Acid glycoprotein concentrations were significantly different at time 0800 on d 1 and were used as covariates. Behavior data were normalized by log transformation for analysis and are presented as percentage of observation before transformation. Behavior data within heifer were analyzed using a paired T-test analysis (27).

RESULTS

Physiological Data

Acute phase proteins. Plasma haptoglobin concentrations were significant for treatment \times time interactions, but no overall treatment effect was detected (Figure 1a). Further analysis of treatment means showed that haptoglobin was not altered by banding. Banded groups tended to have increased haptoglobin concentrations on h 120 to 168. However, a significant increase in haptoglobin was evident by h 168 and 240 (24 and 72 h postdocking), but only in D heifers. α_1 -Acid glycoprotein concentrations (Figure 1b) were significant for treatment and block main effects. These data were analyzed using prebanding concentrations as a covariate because of the significant difference among treatments prebanding. Docked heifer mean AGP concentrations were lower than those of the C and DL heifers. No treatment \times time interactions were detected.

Tumor necrosis factor- α . Tumor necrosis factor- α concentrations showed a main effect for time, but no treatment or block effects or treatment \times time interactions (Figure 2).

Cortisol. Plasma cortisol concentrations were quite variable and the highest peaks of cortisol concentration occurred in the control group (Figure 3). Main effects included time and block effects with a trend ($P = 0.09$) for treatment \times time effects. Analysis of treatment means showed that cortisol concentrations from D heifers were less at 12 h postbanding than the C group. A similar trend ($P = 0.06$) occurred at 48 h postbanding. No treatment differences were observed after the docking procedure by 144 h postbanding.

Leukocyte phenotypes. CD4⁺ and CD8⁺ lymphocyte analysis detected only a time effect (Table 2). CD4⁺ lymphocytes increased for all treatments after the first sampling. CD8⁺ cells also increased in all heifers at 24 h postbanding. A block effect was evident for CD8⁺ but not for CD4⁺ lymphocytes. Similarly, the CD4:CD8 ratio exhibited treatment, time, and block main effects, but no interactions were detected. The control heifers had the highest ratio and the docked heifers had the lowest

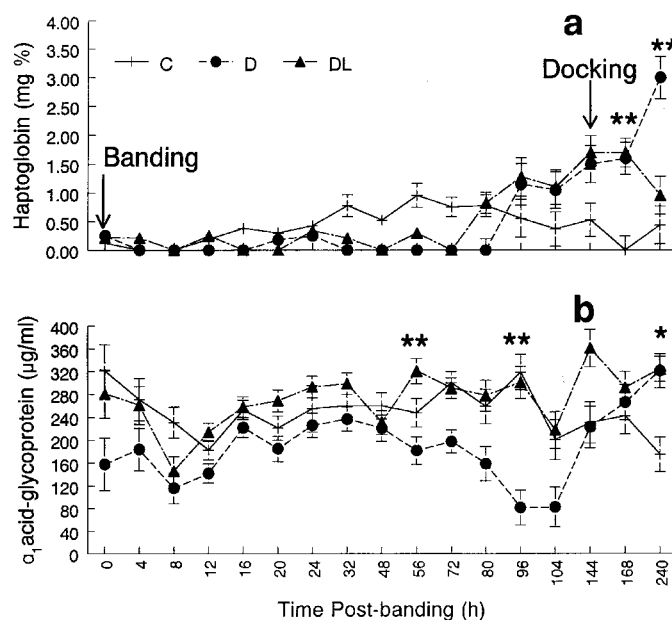


Figure 1. Plasma haptoglobin (a) prior to and following banding (0 h) and docking (144 h) of control (C), docked (D), and docked plus subcutaneous lidocaine (DL) first calf heifers. Significant treatment by day interactions were detected ($P < 0.05$). **Means within the same time with different superscripts differ ($P < 0.05$). Plasma α_1 -acid glycoprotein (b) before and after banding (0 h) and docking (144 h) of control, docked, and docked plus subcutaneous lidocaine heifers. Significant treatment and block main effects were detected ($P < 0.05$). *Means within the same time with different superscripts differ ($P < 0.10$). Day 0 values were used as covariates for the subsequent time analyses.

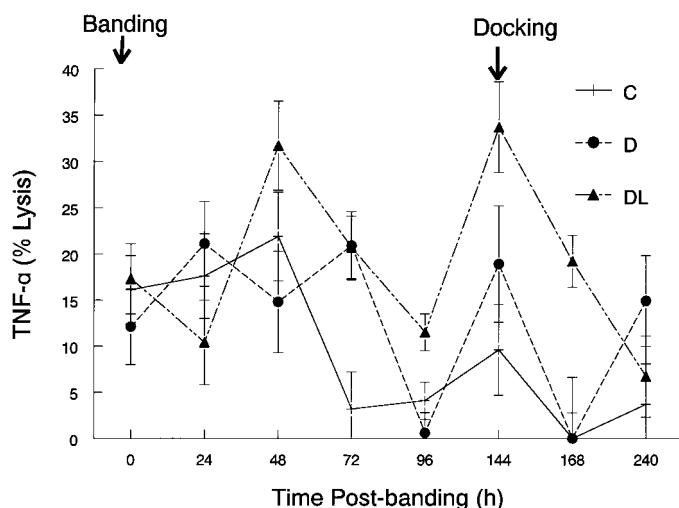


Figure 2. Plasma tumor necrosis factor- α (TNF- α) biological activity prior to and following banding (0 h) and docking (144 h) of control (C), docked (D), and docked plus subcutaneous lidocaine (DL) heifers. Significant block main effect was detected ($P < 0.05$).

ratio. The percentage of lymphocytes positive for the $\gamma\delta$ T-cell did not show treatment main effects, but a time effect and treatment \times time interactions were detected. The $\gamma\delta$ marker tended ($P = 0.06$) to increase at d 168 h (24 h postdocking) for D heifers.

Behavioral Data

Behavioral comparisons were made between pre- and postbanding observations and between pre- and post-

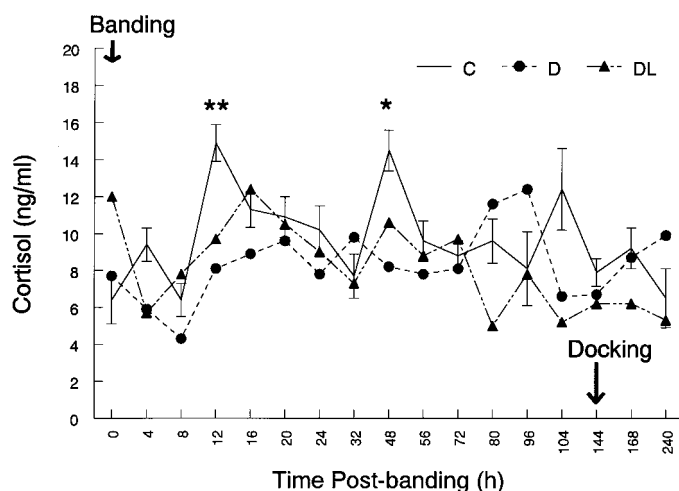


Figure 3. Plasma cortisol prior to and following banding (0 h) and docking (144 h) of control (C), docked (D), and docked plus subcutaneous lidocaine (DL) heifers. Significant treatment and block main effects were detected ($P < 0.05$). Trends ($P = 0.09$) for treatment by day interactions were detected. **Means within the same time with different superscripts differ ($P < 0.05$). *Means within the same time with different superscripts differ ($P < 0.10$).

docking observations. Time spent eating was altered by both banding and docking (Table 3). The docked groups spent more time eating ($P < 0.01$) after banding and less time eating after docking ($P < 0.05$). Data are presented as a percentage of observations (excluding time out-of-view, time out-of-pen, and dark). No differences were found in lying, standing, walking, drinking, head-to-tail, or grooming behaviors.

DISCUSSION

Interpreting behavioral changes associated with pain has not been simple. Method and timing of tail-docking in lambs have raised controversial interpretations of the findings. Shutt et al. (28) concluded that surgery was less stressful than rubber rings based on behavioral observations and β -endorphin release. The conclusions of these authors as to the meaning of increased activity with banding and decreased activity with surgery were questioned by Barnett (2), demonstrating the need for greater understanding of the meanings of behavioral changes. However, behavior of lambs has been used in conjunction with physiological measures as a good indicator for recognition and assessment of pain (19). When lambs were tail-docked without castration, the severity and duration of pain indices was half that of the lambs that were docked and castrated at the same time (18). A series of studies on tail-docking and castration in lambs, showed the response of lambs to tail-docking alone are similar to lambs that were castrated and tail-docked together, but the responses were of lesser magnitude and duration (19). That study (19) and two other by the same authors (11, 12) showed that a rubber ring with a Burdizzo clamp created the fewest behavioral signs and least cortisol response in 5- and 21-d-old lambs. These authors also noted that younger lambs had more abnormal behavior, but less restlessness, and increased cortisol. In contrast, Dinnis et al. (4) did not see differences in cortisol responses in lambs docked with rubber rings only or rubber rings and castration clamps. Differences in the clamp design were thought to be the difference in results.

Our research showed little behavioral or physiological effects of banding on adult cattle. One aspect of the banding procedures that explains differences in pain responses is proper band placement (between vertebrae). Wilson (31) demonstrated the importance of proper band placement to prevent unnecessary swelling. Petrie et al. (20) reported that a few of the 3- to 4-mo-old calves that they observed exhibited protracted tail shaking and vocalization following application of a rubber band for tail-docking, suggesting there are two response levels to rubber ring tail-docking in this age of calves. Through subjective measures (presence with

Table 2. Peripheral blood mononuclear cell phenotypes of cows that were not docked (control C), docked (D), and docked with subcutaneous lidocaine (DL). CD4⁺ lymphocytes were only significant for main effect of time. CD8⁺ lymphocytes were significant for main effects of block and time. CD4:CD8 ratios were significant for block, treatment, and time main effects. Gamma-delta T-cells were significant for time main effects and treatment by time interactions. $P < 0.05$ for main effects and interactions.

Day	Control	Docked	Dock + Lidocaine	SE	Effect
CD4 ⁺					
0	26.7	25.0	23.5	1.1	Main effect: time
1	28.9	27.2	35.4	1.2	
3	31.0	27.2	32.4	1.3	
7	31.7	32.6	31.0	1.6	
	30.3	28.7	30.7	1.5	
CD8 ⁺					
0	9.9	12.4	12.4	1.1	Main effects: block and time
1	13.3	14.4	17	0.5	
3	11.7	13.9	14.3	0.5	
7	12.3	12.3	13.8	0.6	
10	10.4	12.0	12.0	0.6	
CD4:CD8					
0	2.81	2.15	2.09	0.04	Main effects: block, treatment, and time
1	2.27	1.89	2.20	0.08	
3	2.86	1.90	2.39	0.07	
7	2.75	2.78	2.38	0.11	
10	2.98	2.48	2.73	0.08	
$\gamma\delta^+$					
0	13.0	18.3	13.5	1.1	Main effect: time
1	16.3	14.6	16.6	2.6	Interaction: treatment by time
3	21.0	24.1	17.6	1.6	
7	25.0	28.3	18.8	1.5	
10	22.3	25.2	19.0	1.6	

the cows following banding) and objective measures (lack of evidence of vocalization and tail-shaking on video tapes) these two behaviors were not detected in 24-mo-old first calf heifers in our study. In a current study with 3- to 4-wk-old calves, the head-toward-tail and hyperactivity were significantly increased with banding (unpublished data), suggesting an age effect of that pain response in cattle.

The only behavioral change that our data detected was an increase in time spent eating during the week in which the heifers' tails were banded. Eating behaviors

then returned to baseline values when tails were removed. We speculate that the increased eating following banding of the heifers may be a displacement behavior, similar to that of tail-pinched rats who gained weight following pinching (26). Similarly, lambs increased activity and sometimes eating with tail-docking and castration (18). Feed consumption and eating frequency appear to be indicators of mild distress in several species. Cortisol is a measure of acute stress accepted across species. Docking 3- to 4-mo-old calves with a rubber ring, created no more stress (cortisol

Table 3. Behavioral changes pre- and postbanding and pre- and postdocking (tail amputation).

Behavior	(Percentage of observation ¹)			
	Preband	Postband	Predock	Postdock
Lying	51.6	47.2	42.4	43.1
Standing	31.5	31.7	32.2	37.6
Walking	0.4	0.1	0	0
Drinking	3.7	3.5	5.5	4.8
Eating	12.1 ^b	16.6 ^a	17.8 ^a	13.3 ^b
Grooming	0.7	0.9	2.1	1.2
Rubbing pen	0	0	0	0
Interactions	0	0	0	0
Heat-to-tail	0	0	0	0

^{a,b}Means of pre- and postbanding or pre- and postdocking within a behavior with differing superscripts differ ($P < 0.05$).

¹Times the heifer was out of view, out of pen, or not visible because of lighting were excluded. Those times were not different between pre- and postband comparisons.

response) than calves that were handled with simulated docking (21). Likewise, we did not detect cortisol responses in adult dairy cattle to banding or cutting the tail. Most data on tail-docking of lambs showed a slight increase in cortisol concentrations after banding (17, 18), but tail-docking in conjunction with castration greatly increased cortisol concentrations (4, 11, 12, 29). The cortisol response subsided between 6 and 24 h post-banding in 3-mo-old calves (21). These authors also noted the administration of an epidural anaesthetic increased the cortisol response.

The analgesic effect of lidocaine is short lived (24), usually 1 to 2 h. However, other evidence shows altered immune populations with analgesic administration (9, 34). Those changes outlast the analgesic effect as shown here. Because the application of the bands alone induced no detectable behavioral or physiological indicators of pain, we concluded that in adult cattle the administration of the subcutaneous lidocaine at the banding site is unnecessary at the time of banding and too short lived to alleviate later pain from swelling or amputation of the necrotic tail. A similar conclusion was noted by Petrie et al. (21), with an overall conclusion of no benefit for local anaesthetic. Graf and Senn (8) noted that the injection puncture and pressure of liquid at the site resulted in indications of transient stress and acute pain of calves before dehorning. However, Wood et al. (32), showed a beneficial effect of local anaesthesia for 5- to 6-d-old lambs for castration and docking procedures performed together. Interestingly, local anesthetic prevented pain-induced behavior changes in 2-wk-old pigs, but not in 7-wk-old pigs (16). The changes in pain experience, behavioral expression of that pain, and anesthetic and analgesic alleviation of the pain appears to have many age and species-specific aspects.

Other concerns created by tail-docking of dairy cattle are the possibilities of chronic pain and the inability to avoid flies during fly season. New Zealand researchers found increased fly counts and increased fly avoidance behavior associated with tail-docked cows (22, 31). This research was conducted on cows on pasture, so no research is available on the effect of docked tails on fly numbers and fly avoidance behaviors of dairy cows in tie-stall or free-stall housing. The relationship between fly numbers and lack of tails has not been established, therefore excellent fly control is necessary for tail-docked cows until further research substantiates reduced flies on docked cows. Chronic pain has been suggested to result from formation of neuromas in amputated limbs, tails, and beaks (6, 7). A group of Australian researchers has identified neuromas in the stump of docked dairy cows (J. L. Barnett, 1997, personal communication). Recently, the hypothesis of neuroma formation as the sole source of phantom pain has been

questioned by neuropsychologists. Another hypothesis (30) is that synaptic long-term depression in the central nervous system (anterior cingulate cortex) could play a role in enhanced neuronal responses to somatosensory stimuli after amputation. This cortical reorganization in the rat somatosensory cortex occurs during learning as it does following amputations. In humans phantom limb pain is highly associated with human cognitive abilities such as self-awareness (23). In this framework we have little information on whether or not chronic pain results from production practices such as tail-docking. These questions warrant further research to clarify the life-long effect of tail-docking on dairy cows.

CONCLUSIONS

Tail-banding had little effect on cortisol, immune measures, and behavior. Removal of the necrotic tail at 144 h postbanding, increased plasma haptoglobin concentrations. This is consistent with the responsiveness of haptoglobin to tissue damage. α_1 -Acid glycoprotein appears to increase in docked heifers by 240 h postbanding. The effect of lidocaine was evident in lymphocyte phenotype and $\text{TNF-}\alpha$. The $\text{TNF-}\alpha$ response was probably related to the increase in lymphocyte population following banding with lidocaine. In adult cattle, behavior and cortisol stability suggest anesthetic is not necessary for acute pain at banding, but pain management may be beneficial following banding.

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